## Develop New or Improved Methods for Diagnosing Disease and Disability

**IN THIS SECTION:**



**SCIENCE ADVANCES**



**SCIENCE CAPSULES** (page 396)

## More Evidence that Methamphetamine Produces Long-term Damage to Human Brain Cells

*Background:* In recent years, abuse of methamphetamine has been increasing and spreading across the United States. Methamphetamine is a powerfully addictive drug that profoundly affects the central nervous system, and recent research has suggested that it may cause long-term injury to the brain. For example, a study in rhesus monkeys showed that the neurotoxic effects of methamphetamine persist up to four years after the last exposure to the drug. Studies in rodents have shown that methamphetamine is toxic to certain brain cells that contain the neurotransmitters dopamine and serotonin. Furthermore, recent studies of human methamphetamine abusers show a decrease in dopamine transporter binding that may indicate neurotoxicity. Results from two recent studies using brain imaging techniques appear to provide direct evidence that methamphetamine is neurotoxic in humans.

*Advance:* Researchers have now clearly demonstrated that methamphetamine can cause long-lasting injury to the human brain. In a recent study, human subjects who had used methamphetamine for at least five years and had been abstinent an average of 4.25 months and for as long as 21 months were studied using a brain imaging technique that allows researchers to measure concentrations of certain biological markers that are associated with damage to neurons. One marker, N-acetylaspartate (NA), provides a measure of nerve cell viability. In abstinent, long-term methamphetamine abusers, NA was reduced in several brain areas, indicating that neurons in these regions have been damaged. Two other markers, myoinositol and choline-containing compounds were elevated in the frontal cortex of the former methamphetamine abusers. These substances are present in glial cells, a special type of cell that surrounds and supports neurons. When neurons are damaged or die, glial cells proliferate, which results in an increase in myoinositol and choline-containing compounds. The increase in these substances seen in the present study along with the reductions of NA in the brains of former methamphetamine abusers indicates that methamphetamine can cause injury to neurons that is long-term and perhaps even permanent.

*Implications:* This study provides direct cellular evidence in humans that methamphetamine causes damage to nerve cells that persists even after drug use has been discontinued. This damage may explain persistent abnormal behaviors, such as violence, psychoses, and personality changes, which are often observed in former abusers months and even years after they have stopped using methamphetamine. These results have important implications for the treatment of methamphetamine addiction. They indicate that research efforts should focus not only on the development of treatments for methamphetamine addiction, but also on reversing any damage that may have occurred as a result of abuse. Clearly, sustained education and prevention efforts continue to be a critical component of addressing addiction to methamphetamine, as well as other drugs.

Ernst T, Change L, Leonido-Yee M, Speck O: Evidence for long-term neurotoxicity associated with methamphetamine abuse: A $^{1}$H MRS study. Neurology, 54(6):1344-9. 2000.

## Abuse of Stimulants Increases the Risk of HIV-related Brain Damage

*Background:* In the United States, behavior associated with drug abuse is the single largest factor in the spread of HIV infection, with half of all new HIV infections occurring among injecting drug users. Given that many drugs of abuse can also cause damage to the brain, researchers are investigating whether these drugs can further increase HIV-related damage to the brain.

*Advance:* Using a brain imaging technique known as magnetic resonance spectroscopy (MRS) researchers found that stimulant addiction may augment the damage to the brain that is seen with HIV infection. The researchers used MRS to measure levels of certain brain substances that are associated with the degeneration and death of neurons in patients who were HIV positive and stimulant dependent; in patients who were HIV positive and did not use drugs; in patients that were HIV negative and stimulant dependent; and in a group of normal controls. Those who were HIV positive and stimulant dependent had significant differences in brain levels of several substances that are associated with damage to neurons when compared with normal subjects and also with the other two patient groups.

*Implications:* The current study suggests that brain deficits may be more pronounced in HIV positive individuals who are also addicted to stimulants. Currently, it is not known whether these deficits are due to reversible brain changes or irreversible nerve cell death. This study underscores the importance of fully characterizing brain changes that occur as a result of HIV infection and drug addiction, as a means of developing treatments that can address any brain damage that occurs. These studies also emphasize the need for continued prevention efforts aimed at reducing drug addiction and the spread of HIV.

Taylor MJ, Alhassoon OM, Schweinsburg BC, Videen JS, Gran I, and The HNRC Group: MR spectroscopy in HIV and stimulant dependence. Journal of the International Neuropsychological Society, 6(1):83-5. 2000.

## Pinpointing Genes for Kidney Disease of Diabetes

*Background:* Diabetes is the most common cause of end-stage kidney disease in the U.S. In diabetes patients, even modest amounts of protein in the urine increase the risk of progressive kidney disease as well as cardiovascular complications. Family studies have established that the susceptibility to diabetic kidney disease runs in families; not all diabetics develop kidney disease, even after many years at risk, and the vulnerability to disease appears to be under control of genetic factors. The Pima Indians of Arizona are known to have a particularly high prevalence of type 2 diabetes and diabetic kidney disease.

*Advances:* Two recent studies examined genetic susceptibility to kidney disease. One study – the first of its kind – included 96 families having over 600 people with and 600 people without type 2 diabetes. Significant differences were found in the degree of protein excreted in the urine in families with and without diabetes. In addition, in families having both diabetic and nondiabetic individuals, the level of urine protein of the diabetic individuals correlated directly with the level, within the "normal" range, of urine protein in the nondiabetic individuals. While the presence of diabetes adds to the probability of higher urine protein, and therefore a greater risk for progressive kidney disease, this effect interacts with the genetic factors that cause susceptibility to kidney disease that are not linked to diabetes. A second study included over 2,000 Pima Indians from 715 families. These studies showed that there is one major genetic determinant associated with the development of diabetic kidney disease in this population, and raised the possibility that this gene acts as a single dominant determinant. Contrary to previous studies, the duration of diabetes did not predict whether or not individuals carried the genetic susceptibility region. The authors propose that other genetic determinants with more minor effects are likely involved as well, but narrowing down the number of genes is valuable for the eventual goal of identifying the susceptibility genes.

*Implications:* Understanding the heritable components of complex genetic diseases such as kidney disease and diabetes is proving to be a major challenge. These studies provide evidence that protein in the urine is a good marker of disease susceptibility, independent of additional factors that may increase the risk of kidney disease. Furthermore, the evidence that one major gene may increase susceptibility to the kidney disease of diabetes, at least in the Pima Indian population, increases confidence in the feasibility of eventually identifying that gene. Other genes may have a weaker but a significant role in susceptibility. In addition, the studies point out that different analytic strategies may provide different associations with complications in complex diseases. These findings help to establish a foundation for future genetic analyses of kidney disease, particularly that caused by diabetes, in Caucasian and Pima Indian populations. [secondary – prevention]

Fogarty DG, Hanna LS, Wantman M, et al: Segregation analysis of urinary albumin excretion in families with type 2 diabetes. Diabetes, 49(6):1057-63. 2000.

Imperatore G, Knowler WC, Pettitt DJ, et al: Segregation analysis of diabetic nephropathy in Pima Indians. Diabetes, 49(6):1049-56. 2000.

## Cost-Effectiveness Studies of Kidney Dialysis and Transplantation

*Background:* When the kidneys fail and cease to remove waste products from the blood, patients require renal replacement therapy, either dialysis or a renal transplant, to stay alive. For most people the preferred form of renal replacement therapy is a kidney transplant, because generally this results in less disability than dialysis and a longer life expectancy. Dialysis is expensive, costing nearly $11 billion in 1997. Currently, two thirds of dialysis patients receive their treatment in for-profit centers.

There are about 40,000 patients in the U.S. waiting for a kidney transplant. The economic cost associated with the tissue matching of donor kidneys with recipients is an unresolved issue. However, good tissue matching improves both short-term and long-term graft survival in local allocation programs. It is unclear whether this is the case in national programs.

*Advance:* Scientists examined the effect of for-profit ownership of dialysis facilities on patient survival and the likelihood that patients would be referred for possible kidney transplantation. Using data from the NIH-supported United States Renal Data System, they found that patients treated at a for-profit, as opposed to a not-for-profit, center had a 20% higher mortality rate and a 26% lower rate of placement on waiting lists for a transplant.

Another team of scientists analyzed the economic costs of transplanting well matched versus less well-matched kidneys. They found that Medicare payments for the three years after transplantations were about $60,000 for each individual in the well-matched group of patients, but nearly $81,000 for persons in the less well-matched patients – a 35% difference in cost. Their analysis of local versus national allocation programs indicated that well-matched transplants resulted in savings within local, but not national, programs owing to deterioration of organs transported nationally.

*Implications:* The studies are important examples of how research can contribute to development of a more cost effective and equitable health care system. The study of for-profit and not-for-profit dialysis centers provides important data about the differences in patient care. This information may be used to develop quality assurance standards for the U.S. end-stage renal disease program. The study of the economic impact of tissue matching in transplantation suggests that cost benefits may be accrued from good tissue matching within local allocation programs.

Garg PP, Frick KD, Diener-West M, Powe NR: Effect of the ownership of dialysis facilities on patients' survival and referral for transplantation. The New England Journal of Medicine, 341(22):1653-60. 1999.

Schnitzler MA, Hollenbeak CS, Cohen DS, et al: The economic implications of HLA matching in cadaveric renal transplantation. The New England Journal of Medicine, 341(19):1440-6. 1999.

## Deciphering the Genetics of Type 2 Diabetes

*Background:* Type 2 diabetes is the predominant form of diabetes in the United States. It is characterized by insufficient levels of insulin and by resistance to the action of insulin. More than one genetic alteration is probably necessary for development of the disease. In addition, a different combination of genetic factors may play a role in different populations. The genes involved in some relatively rare subtypes of type 2 diabetes, such as Maturity Onset Diabetes of the Young (MODY), have been identified, but seem to play a relatively minor role in the more common form of type 2 diabetes.

*Advances:* Several groups of investigators are searching for genes that may confer susceptibility to type 2 diabetes. Genome-wide screens have led to the localization of a susceptibility gene on chromosome 2 for type 2 diabetes in a Mexican American population. This candidate gene, designated NIDDM1, interacts with a gene on chromosome 15 to increase diabetes susceptibility. Recent studies describe the discovery of a gene localized in the NIDDM1 region that shows an association with type 2 diabetes in Mexican Americans and in two Northern European populations. This proposed diabetes susceptibility gene codes for a protein called calpain 10 (CAPN10). Individuals in these populations with a certain combination of genetic markers have approximately a threefold increased risk of type 2 diabetes. The genetic variation in CAPN10 appears to affect the risk of type 2 diabetes, suggesting that susceptibility is not attributable to a single variation, but rather to multiple variations whose collective effects are not easily predicted. The presence of CAPN10 in the insulin-producing pancreatic islet cells, muscle and liver suggests that it could affect insulin secretion, insulin action and glucose production by the liver, each of which is altered in type 2 diabetes. Taken together, these results demonstrate new pathways that may be involved in the regulation of blood glucose and the development of diabetes, and suggest that other calpain genes may affect susceptibility to diabetes. One of these, CAPN3, is located in the region of chromosome 15 containing the susceptibility area that interacts with NIDDM1 and will be a target of future investigations.

Recently, another locus (NIDDM2) for type 2 diabetes was identified on chromosome 12 in an isolated Finnish cohort. Similar studies have failed to confirm that this locus contributes to type 2 diabetes in other populations. A study in the U.S. population found evidence for a link between diabetes and chromosome 12q, but this locus is closer to the centromere – the primary constriction point in the chromosome – than NIDDM2. In addition, there is strong evidence for linkage in families with diabetes characterized by a poor insulin response. Another study in American populations (GENNID) also found evidence for a diabetes susceptibility locus in the same region on chromosome 12.

Other investigators have described two mutations in the NEUROD1 gene, which are associated with the development of type 2 diabetes. The protein encoded by this gene functions as a regulatory switch for the development of the portion of the pancreas that secretes insulin and glucagon. In mice carrying two disrupted copies of the gene, pancreatic islet development is abnormal and overt diabetes develops,

due in part to the insulin gene's inadequate expression – the process by which a gene's coded information is activated. Investigators screened 94 patients with type 2 diabetes for mutations in NEUROD1 and identified 2 families with mutations in this gene. The researchers hypothesized that the development of type 2 diabetes in carriers of either of these two mutations results from a disruption of gene activity in the islets due to deficient binding of NEUROD1 or binding of an inactive NEUROD1 to targets in the pancreatic islet cells.

Investigators in collaboration with French investigators have demonstrated that mutations in the insulin promoter factor-1 (IPF-1 also known as PDX1) gene may be related to the development of type 2 diabetes. IPF-1 is critical for embryonic development of the pancreas and for regulation of endocrine pancreas-specific genes in adults. Some patients with mutations in IPF-1 develop MODY. Researchers examined the IPF-1 gene for mutations in 64 unrelated type 2 diabetes patients of French ancestry and uncovered three novel IPF-1 mutations. This is the first reported evidence that IPF-1 may represent a diabetes-predisposing gene in a portion of the common form of type 2 diabetes cases.

*Implications:* Discoveries of genes that may predispose to the development of type 2 diabetes have opened up important new avenues of research into the underlying causes of diabetes, such as those controlling signaling molecules and transcription factors, and their targets. Recent advances in determining the genetic makeup of an individual, gene mapping and completion of the human genome will aid in our understanding of the genetic development of diabetes. This information, in turn, will lead to improved diagnosis, treatment and prevention, thereby helping to stem or even reverse the increasing incidence of this devastating disease.

Horikawa Y, Oda N, Cox NJ, et al: Genetic variation in the calpain 10 gene (CAPN10) is associated with type 2 diabetes mellitus. Nature Genetics, 26(2):163-75. 2000.

Malecki MT, Jhala US, Antonellis A, et al: Mutations in NEUROD1 are associated with the development of type 2 diabetes mellitus. Nature Genetics, 23(3):323-8. 2000.

Bektas A, Suprenant ME, Wogan LT, et al: Evidence of a novel type 2 diabetes locus 50 cM centromeric to NIDDM2 on chromosome 12q. Diabetes, 48(11):2246-51. 1999.

Hani EH, Stoffers DA, Chevre JC, et al: Defective mutations in the insulin promoter factor-1 (IPF-1) gene in late-onset type 2 diabetes mellitus. Journal of Clinical Investigation, 104(9):41-8. 1999.

## Mutations in Nuclear Protein Linked to Lipodystrophy

*Background:* Lipodystrophy refers to a group of conditions that result in defective metabolism of fat, causing the reduction or absence of fatty (adipose) tissue. The lipodystrophies may be acquired or inherited and both the anatomic location and degree of fat loss vary among the different disorders. Patients with familial partial lipodystrophy (FPLD), Dunnigan variety, have normal adipose tissue distribution during childhood. With the onset of puberty, these individuals lose subcutaneous fat from the extremities, trunk and gluteal regions of the body, but excess fat becomes deposited in the face, neck and back. These conditions pose a metabolic threat, as they are often accompanied by insulin resistance, elevated levels of blood lipids (a group of fatty acids found in all living things) and vascular disease. In addition, affected patients usually develop type 2 diabetes. Previously, researchers localized the gene for FPLD to a certain region on chromosome 1. Other investigators have linked mutations in the gene that directs the production of the protein lamin A/C, a component of the nuclear envelope – the double-layered membrane enclosing the nucleus of a cell – with a form of muscular dystrophy and, most recently, FPLD. The lamins themselves have been implicated in many functions, including the mediation of DNA replication, or the process whereby DNA is copied. To further evaluate the role of this gene in FPLD, researchers studied affected family members in a cohort of 15 families.

*Advance*: Researchers detected four independent mutations in this gene in members of 14 families. All of the alterations resulting in FPLD occur within a particular region of the lamin A/C protein. Mice with a targeted deletion of this gene not only develop a form of muscular dystrophy, but also lack distinguishable white fat, which serves as a valuable source of energy. Collectively, these data imply that mutations within this region of the lamin A/C protein are involved in one or more activities required by fat cells in specific tissue beds. It has been hypothesized that in FPLD, loss of fat cells affects insulin sensitivity through reduced levels of "adipocyte-derived circulating factors" such as leptin, a hormone produced by fat cells that regulates food intake and energy metabolism. Researchers have generated a mouse model that lacks white fat and is insulin resistant and leptin deficient. This animal also has type 2 diabetes, a disease that involves altered fat cell size and deposition, and insulin resistance. Researchers found that treating this model with leptin did not correct the insulin resistance associated with type 2 diabetes. They concluded that leptin deficiency may contribute to the insulin resistance of generalized lipodystrophy, but it is not the sole nor principal cause of insulin resistance in severe forms of this disease.

*Implications:* These studies confirm earlier findings that mutations in the gene that codes for the nuclear envelope protein lamin A/C are inherited with FPLD. This is the first gene known to be altered in the lipodystrophies, broadening the range of disorders for which components of the nuclear envelope may be considered as putative candidates. Further investigation of the lamins and the pathways in which they act may also provide clues into complex metabolic disorders such as type 2 diabetes by dissecting out new pathways that regulate fat cell mass and insulin sensitivity.

Speckman RA, Garg A, Du F, et al: Mutational and haplotype analyses of families with familial partial lipodystrophy (Dunnigan variety) reveal recurrent missense mutations in the globular C-terminal domain of lamin A/C. American Journal of Human Genetics, 66(4):1192-8. 2000.
Gavrilova O, Marcus-Samuels B, Leon LR, et al: Leptin and diabetes in lipoatrophic mice. Nature, 403(6772):850-1. 2000.

## Common Polymorphisms of the GJB2 (Connexin 26) Gene and Hereditary Deafness

*Background:* Mutations in the GJB2 gene cause a significant proportion of recessive deafness in many ethnic populations. GJB2 is thought to be important for communication between adjacent cells in the inner ear. Many variants, or polymorphisms, of GJB2 are common in the population and it is thought that they can cause deafness. One of the more common polymorphisms, called M34T, is known to significantly decrease the intercellular communication activity of GJB2, and M34T is widely thought to cause deafness.

*Advance:* A study of a family with hereditary deafness in which the M34T variant is not associated with deafness suggests that M34T, as well as some other polymorphisms, may not be deafness-causing variants of GJB2 even if they affect molecular function.

*Implications:* This finding has important implications for understanding how GJB2 functions in the inner ear, as well as for counseling patients with GJB2 polymorphisms such as M34T.

Griffith AJ, Chowdhry AA, Kurima K, et al: Autosomal recessive nonsyndromic neurosensory deafness at DFNB1 not associated with the compound-heterozygous GJB2 (Connexin 26) genotype M34T/167delT. American Journal of Human Genetics, 67(3):745-9. 2000.

## The Neurobiology of Depression and Suicide

*Background*: Major depression is a serious illness that affects approximately 5% of the population and carries a significant risk of suicide. Both depression and suicide share several features including altered function of the neurotransmitter serotonin, one of the brain's chemical messengers; a diminished number of serotonin transporter sites, which are tiny pump-like components on certain neurons that play an important role in regulating the amount of serotonin in the synapse; and changes in both cerebrospinal fluid and platelets. Despite these similarities suicide may be – at least in some cases – separable from a specific psychiatric diagnosis. This leads to two questions: (1) Do serotonergic abnormalities occur in different brain regions in persons with major depression and individuals at high risk for suicide? And (2) What factor(s) are responsible for the alterations in serotonergic function? A team of NIH-funded investigators has spent almost two decades addressing these issues. They now hypothesize that the predisposition for impulsive aggression and suicide relates to brain dysfunction specific to one area of the prefrontal cortex, while depression involves more widely dispersed brain circuitry.

*Advance*: These investigators recently examined serotonin transporter molecules in the prefrontal cortex in *postmortem* brains. In their analysis, they were looking for evidence of a polymorphism, or variation, of the gene – called the serotonin transporter gene promoter – that is involved in producing the transporter molecule. They found that although the transporter is expressed, or produced, at lower than normal levels in specific areas of the cortex of suicide victims, this does not correlate with the presence of the promoter polymorphism. In contrast, depressed patients demonstrated, post-mortem, wide-spread reduction of transporter throughout the prefrontal cortex, and these changes correlate with the occurrence of the promoter polymorphism.

*Implications:* Subtle differences in the expression of molecules that mediate the function of a major neurotransmitter in the brain are important clues to disease processes. These findings suggest that suicide and major depression may be separate brain disorders, each with a distinct mechanism. Clearly there is overlap between the disorders because major depression is a significant risk factor for suicide. However, the findings demonstrate that fine-tuning the understanding of subtle molecular processes is an effective strategy for refining diagnostic specificity. Most importantly, these molecular processes offer novel targets for new treatments and possible preventive interventions.

Mann JJ, Huang Y, Underwood MD, et al: A serotonin transporter gene promoter polynorphism (5-HTLPR) and prefrontal cortical binding in major depression and suicide. Archives of General Psychiatry, 57(8):729-38. 2000.

## Evidence for a Genomic Region Containing a Schizophrenia Susceptibility Gene

*Background:* Schizophrenia is a persistent, debilitating mental disorder that afflicts about 1% of the population. Prominent features of the illness include psychotic symptoms (delusions and hallucinations), thought disturbances, and social withdrawal. Family, twin, and adoption studies have consistently demonstrated that schizophrenia has high heritability. It also is clear that the disorder does not follow a simple mode of inheritance within families, which would suggest responsibility of a single gene. Rather, it is likely that multiple genes of small relative effect and environmental factors likely interact in a complex way to produce susceptibility to the disorder.

*Advance*: Twenty-two Canadian families were recruited to participate in this study. Each extended family contained an average of four persons with clinical diagnoses of schizophrenia or schizoaffective disorder, quite narrowly defined. Using DNA drawn from blood samples, 288 subjects were genotyped using 381 genetic markers. Genotyping was done by the Center for Inherited Disease Research, which was established as a joint effort of eight NIH institutes through a contract to Johns Hopkins University. The analysis yielded evidence supporting a linkage of schizophrenia to a small region on chromosome 1 (1q21-q22). A *lod* score is a mathematical formulation that describes the likelihood that an apparent genetic linkage is true, and not simply a chance result. In this instance, a *lod* score of 6.5 was obtained for linkage to this chromosomal region, which significantly exceeded minimum *lod* score criteria set by the researchers in advance.

*Implications*: The identification of a susceptibility gene would represent a major step in understanding schizophrenia. Although this study does not implicate a specific gene that produces susceptibility, it does implicate a demarcated region on chromosome 1. The magnitude of the statistical evidence and clear localization to a relatively small region should facilitate efforts to positionally clone this suspect gene, an opportunity that adds urgency to the essential next step – confirming this finding in another independent sample of families. Identification of this and other susceptibility genes, and continuing study of complex gene-gene and gene-environment interactions are setting the stage for advances in diagnosis, treatment, and, ultimately, prevention of this disorder.

Brzustowicz LM, Hodgkinson KA, Chow EWC, Honer WG, Bassett AS: Location of a major susceptibility locus for familial schizophrenia on chromosome 1q21-q22. Science, 288(5466):678-82. 2000.

## Utilization of Mitochondrial DNA Mutations for Cancer Detection Using Body Fluids

*Background:* Mitochondria are cellular organelles which are our cell's power sources. There are hundreds of mitochondria in each cell, and they each have multiple copies of their own genome. The majority of these copies are identical. Mitochondria are thought to be involved in tumorigenesis and to play a role in apoptosis, or programmed cell death.

*Advance:* Researchers found that the majority of tested bladder, lung, and head and neck tumors had acquired mutations in their mitochondrial DNA. Several of the tumors had multiple mutations suggesting an accumulation of mitochondrial DNA damage. Mutations were readily found in most of the saliva samples obtained from head and neck cancer patients.

*Implications:* The study of mitochondrial DNA mutations should lead to powerful and very sensitive molecular markers for use in the non-invasive detection of cancers using body fluids.

Fliss MS, Usadel H, Caballero OL,et al: Facile detection of mitochondrial DNA mutations in tumors and bodily fluids. Science, 287(5460):2017-9. 2000.

## Use of Structural Magnetic Resonance Imaging to Predict who will get Alzheimer's Disease

*Background:* In this study, magnetic resonance imaging (MRI) measurements were used to determine whether cognitively normal older persons and persons in the very early phase of Alzheimer's disease (AD) could be identified before they developed clinically diagnosed AD. Normal subjects and those with mild memory difficulty received an MRI scan at baseline and were followed annually for three years to determine who subsequently met clinical criteria for AD. Previous research had indicated that measurements of hippocampal volume (a brain region involved in memory) could identify some cases of AD before a patient met clinical criteria for dementia, but in this study the researchers were seeking to improve accuracy by honing in on other select areas of the brain involved at an earlier stage in the disease process. The investigators looked at differences in volume in a number of areas, focusing on the entorhinal cortex and the banks of the superior temporal sulcus, both involved in memory, and the anterior cingulate, which affects "executive" functions such as organizing, planning, and switching back and forth among tasks and ideas.

*Advance:* The researchers found that they could identify people who would develop AD over time based on measurements of these brain regions. The MRIs were 100 percent accurate in discriminating between the participants who were normal and those who already had mild AD. They were 93 percent accurate in discriminating between participants who were normal and those who initially had memory impairments and ultimately developed AD; the entorhinal cortex in the case of the people "converting" to AD had about 37 percent less volume than the entorhinal cortex of those who remained normal, probably reflecting a loss of brain cells. Other comparisons showed a relatively high accuracy rate as well, although it was more difficult to distinguish the people who continued to have memory problems but did not progress to AD from those who eventually converted to AD.

*Implications:* This study offers evidence establishing the involvement of specific areas of the brain in the underlying early pathology of AD. It also suggests that, by zeroing in on these areas, it may be possible to use already available imaging techniques to better identify people at greatest risk and those for whom early treatment could make a difference. However, the MRI technique will need to be further refined and validated before it can be used in everyday practice by practicing physicians, and more research in several areas will need to be done, including follow-up of patients over a longer period of time to more precisely gauge the predictive value of the MRIs.

Killiany RJ, Gomez-Isla T, Moss M, et al: Use of structural magnetic resonance imaging to predict who will get Alzheimer's disease. Annals of Neurology, 47(4):430-9. 2000.

## Rapid Inexpensive Method for Detecting Tuberculosis in Patient Sputum Samples

*Background:* Each year there are an estimated eight million new cases of clinical tuberculosis, and three million deaths due to the bacterium that causes the disease. Most cases occur in developing countries with resource-poor settings and restrained abilities to use conventional tests because of high costs and limiting equipment. Because of these limitations, drug susceptibility testing is rarely performed in developing countries despite the rapidly rising rates of multiple-resistance strains.

*Advance*: A team of U.S. and Peruvian researchers supported by the International Training and Research Program for Emerging Infectious Diseases developed a rapid and reliable method for detecting tuberculosis to be used by health officers in developing countries. The test, called "microscopic observation broth-drug susceptibility assay," can detect tiny amounts of the tuberculosis bacteria (mycobacterium tuberculosis) in patient sputum samples within 9 days compared with 3-4 week results by traditional culture methods. Tuberculosis bacterial growth in inexpensive liquid media is observed as characteristic strings and tangles with a simple light microscope. This method can also be used to determine whether a particular strain of M. tuberculosis is resistant to any drug without the use of radioactive isotopes or fluorescent indicators

*Implications*: Use of this rapid, inexpensive and sensitive method should improve the capability to detect and properly treat tuberculosis in developing country health facilities.

Caviedes L, Lee T, Gilman R,et al: Rapid, efficient detection and drug susceptibility testing of mycobacterium tuberculosis in sputum by microscopic observation of broth cultures. The tuberculosis working group in peru. Journal of Clinical Microbiology, 38(3):1203-8. 2000.

## University of Hawaii Identifies First Gene Mutations in a Blood Vessel Disease

*Background:* While relatively rare, occurring in about 1 in 25,000 live births, pseudoxanthoma elasticum (PXE) can have devastating consequences. In PXE, elastin fibers in the matrix or space between cells of the skin, retina, and arteries become calcified and lose their resilience. The disease usually begins as a rash of yellow papules on the skin, and can eventually cause the skin to sag and wrinkle, giving the appearance of premature aging. Rupture of a membrane at the back of the retina can lead to blindness, and premature arteriosclerosis can necessitate heart bypass surgery in patients still in their twenties. Gastrointestinal bleeding from ruptured blood vessels is a frequent cause of PXE-related death.

*Advance*: An international research team headed by the University of Hawaii's Laboratory of Matrix Pathobiology has identified the first mutations responsible for this genetic disease. Discovery of the PXE mutation builds on work by a Harvard team that narrowed the search for the gene to a specific region of Chromosome 16. By comparing genomic DNA from 17 unrelated PXE patients, the Hawaii investigators were able to distinguish the disease-causing gene from among the hundreds of thousands of base pairs in the implicated section of DNA. Comparison of the gene's building-block base pairs turned up several mutations in PXE-affected individuals that were not present in DNA samples from a control group. The PXE gene codes for production of a transport protein that shuttles proteins across cell membranes. The gene has been associated with drug resistance that occurs when drug compounds are not carried across the cell membrane into the cell.

*Implications:* Discovery of the genes responsible for PXE makes it possible to screen for the disorder and implement dietary interventions to lessen the impact of the disease. Studies of this gene and its protein product may also lead to better understanding of how drugs and other compounds can gain access to cells.

Le Saux O, Urban Z, Tschuch C, et al: Mutations in a gene encoding an ABC transporter cause pseudoxanthoma elasticum. Nature Genetics, 25(2):223-7. 2000.

## New Role of Progesterone in Pregnancy Maintenance

*Background:* For a long time, scientists have known that progesterone is essential for maintaining pregnancy; however, little is known about how the hormone works to achieve this goal. In addition, it remains a biological mystery why an embryo, which is half-foreign to the mother's immune system, is not rejected in the uterus during pregnancy. While some explanations have been proposed and tested, no single mechanism has proven to be a required element in this process. This research gives a novel clue and sheds new light on these long-standing issues.

*Advance:* In a recent study, researchers found that progesterone receptors are present in the stromal, or supporting, tissue of the thymus, a gland which produces immune cells called T lymphocytes (T-cells). When animals become pregnant, progesterone is secreted in a large quantity and binds to these receptors. The binding of progesterone to the receptors *reduces* the number of T-cells on the outer layer of the thymus. This is important because the T-cells recognize the foreign antigens of an embryo or fetus that are produced and enter the mother's system when she becomes pregnant. By increasing progesterone secretion during pregnancy, the T-cells that may be toxic to the embryo are removed. The investigators also demonstrated the importance of progesterone receptors by transplanting the thymus tissue from a mouse that lacked these receptors, into a normal mouse. In the experimental mouse, the transplanted thymus tissue did not reduce the amount of toxic T-cells because the stroma had no progesterone receptors. Many of the embryos in the mouse were resorbed as pregnancy progressed.

*Implications:* These observations demonstrate a "progesterone receptor-dependent mechanism" that blocks the very early development of T-cells during pregnancy, and is essential for normal fertility. The new findings provide researchers with a promising new avenue to improve the diagnosis and treatment of frequent miscarriages.

Tibbetts TA, DeMayo F, Rich S, et al: Progesterone receptors in the thymus are required for thymic involution during pregnancy and for normal fertility. Proceedings of the National Academy of Sciences, 96(21),12021-6. 1999.

## Variation in the *HOXA1* Gene Linked to Autism

*Background*: Autism spectrum disorders (ASDs) are severely incapacitating, life-long developmental disabilities associated with severe problems in communication, in social interaction, and in repetitive actions and interests. Autism is one of the most common developmental disorders and occurs in more than one out of every 1000 births. There is strong evidence that ASD has a genetic basis. Scientists have found that mice without two genes, *Hoxa1* and *Hoxb1*, which are critical for early brain development, exhibit characteristics similar to autism. In addition, other research findings support the possible role of these genes in ASD susceptibility; however, the mechanism by which this occurs is still not understood.

*Advance*: Scientists found a region on the human *HOXA1* gene that is associated with ASD. Scientists analyzed the DNA of autistic patients and found that there were coding variations in the *HOXA1* and *HOXB1* genes. Then scientists analyzed the DNA of ASD and non-ASD families and found that the ASD families were more likely to carry and pass on the slightly altered codes in the *HOXA1* region.

*Implications*: These results show that certain codes in the *HOXA1* gene may play a role in making some families and individuals more susceptible to ASD than others. In addition, the results support the theory that autism has a strong genetic basis and is related to a disruption in early brain development. With this new knowledge, scientists can focus specifically on the coding variation in the *HOXA1* gene and learn more about its role in ASD. Furthermore, the findings may enable scientists to eventually develop a method to diagnose ASD much earlier or to prevent its occurrence.

Ingram JL, Stodgell CJ, Hyman SL, et al: Discovery of allelic variants of HOXA1 and HOXB1: genetic susceptibility to autism spectrum disorders. Teratology, 62(6):393-405. 2000.

## Genetic Studies of a Chronic Condition

*Background*: The lymphatic system is a complex system of vessels that run parallel to the circulatory system collecting lymph from the body's tissues and returning it to the bloodstream. Lymph is a pale, yellow fluid containing plasma and white blood cells. In contrast to the circulatory system, which utilizes the heart to pump blood throughout the body, the lymphatic system pumps lymph fluid using the natural contractions of the lymphatic vessels, as well as mechanisms such as muscle contraction and respiratory movement.

Lymphoedema is a chronic, disabling condition of the lymphatic system that results in the swelling of the extremities. Patients with this disorder suffer from recurring local infections, physical impairment, and social stigmatization, and may be at increased risk of developing certain cancers, such as lymphangiosarcoma. Lymphoedemas are generally classified as primary and secondary: primary lymphoedemas occur spontaneously and generally without a preceding disease, whereas secondary lymphoedemas are preceded by stoppage or slowing of lymph movement due to filariasis (infestation with roundworms), surgical or other trauma, malignant disease or high dose radiation. Scientists believe that lymphatic development and function is regulated by a variety of unidentified genes, which, when mutated, may result in lymphoedema.

*Advance:* Recently, researchers found a genetic linkage for primary inherited lymphoedema to vascular endothelial growth factor receptor 3 (VEGFR-3), a chemical binding site on chromosome 5q which is important for activating normal lymphatic vessel formation and function. Now, scientists have also found mutations in the region of VEGFR-3 that is responsible for making the enzyme, tyrosine kinase. This enzyme plays a key role in signaling the growth of new lymphatic vessels. These results indicate that, in some cases, lymphoedema results from a mutation interfering with this critical signaling pathway.

*Implications:* Researchers have been investigating the genetic basis for primary inherited lymphoedema for several years. Despite a general understanding of the disease process that leads to lymphoedema, essentially nothing had been known about the genetic basis of primary lymphoedema prior to this work. Ultimately, these studies will shed light on the developmental biology of the lymphatic system. Furthermore, the recent findings may permit better-informed genetic counseling in affected families, earlier diagnosis and treatment, and the development of more targeted and effective therapies for lymphoedema. These findings also suggest that the variability in how secondary lymphoedema develops following mastectomy for breast cancer, other surgical procedures, or traumatic injury may result from underlying genetic variation in the VEGFR-3 locus. This genetic variation may significantly affect both the severity and timing of the onset of lymphoedema following surgery or injury. [secondary – treatment]

Karkkainen MJ, Ferrell RE, Lawrence EC, et al: Missense mutations interfere with VEGFR-3 signalling in primary lymphoedema. Nature Genetics, 25(2): 153-9. 2000.

## Familial Primary Pulmonary Hypertension Gene Identified

*Background*: Familial primary pulmonary hypertension (FPPH) is a rare but devastating lung disorder characterized by increased pressure in the pulmonary artery. It is associated with structural changes in the blood vessels of the lung that impede blood flow. This leads to an enlarged, overworked heart that is unable to pump enough blood through the lungs and can cause heart failure and death. The disease affects twice as many women as men. Although it can occur at any age, including childhood, affected women are usually of child-bearing age.

*Advance*: Two separate groups of scientists identified defects in a gene associated with FPPH. Both groups identified several mutations in the bone morphogenetic protein receptor 2 gene (BMPR2) in patients with FPPH. The BMPR2 receptor is found on the surface of cells that are responsible for controlling cell growth and are important in lung development. Although these mutated forms of BMPR2 have not been detected in some people with FPPH, healthy individuals have no mutations in the BMPR2 gene. These findings indicate that mutations in the BMPR2 gene, while definitely a leading cause of FPPH, are not the sole factors influencing development of the disease.

*Implications*: The characterizations of the mutations of the BMPR2 gene that lead to FPPH will provide insight into the underlying molecular mechanisms. Additionally, association of this gene with FPPH has implications for sporadic primary pulmonary hypertension, a non-inherited type of pulmonary hypertension that is clinically and pathologically identical to FPPH. Defining the function of the BMPR2 gene should lead to understanding of how the mutations cause pulmonary hypertension and is likely to provide important information for new approaches to patient diagnosis, genetic counseling, clinical screening, and evaluating future therapeutics.

Deng Z, Morse JH, Slager SL, et al: Familial primary pulmonary hypertension (Gene PPH1) is caused by mutations in the bone morphogenetic protein receptor-II gene. American Journal of Human Genetics, 67(3):737-44. 2000.

## Identification of One of the Genes Whose Mutations Cause Usher Syndrome

*Background:* Usher syndrome type 1 is an inherited sensory defect involving profound deafness and vestibular dysfunction, as well as blindness due to progressive retinitis pigmentosa. Usher syndrome is the most common genetic cause of blindness and deafness in Americans.

*Advance:* Scientists supported by the NIH in collaboration with scientists in France have identified the gene whose mutations cause one type of Usher syndrome (Usher syndrome type 1C) which is particularly prevalent among Acadians (descendants of French settlers or Cajuns) in Louisiana. The gene encodes a protein called harmonin (from the Greek word harmonia, meaning, "assembling") that is expressed in sensory cells in the inner ear-the cells responsible for translating sound waves and movement into neural impulses. Identification and study of the mouse version of harmonin revealed that the protein is distributed in the semi-circular canals and cochlea of the inner ear.

*Implications:* These findings will allow for DNA-based diagnosis of Usher syndrome before the deaf individual begins to lose their sight. Such early diagnosis will permit study of the complete progression of retinal degeneration and provide time for possible treatment before the degeneration begins. Also, the isolated harmonin protein can now be used to identify the other proteins with which it associates in the inner ear. Such information could greatly clarify the molecular components that make up the ear's remarkably sensitive apparatus and may clarify the bases for the sensory defects in Usher syndrome and other auditory disorders.

Verpy E Leibovici M, Zwaenepoel I, et al: A defect in harmonin, a PDZ domain-containing protein expressed in the inner ear sensory cells, underlies Usher syndrome type 1C. Nature Genetics, 26(1):51-4. 2000.

## Gene for Cleft Lip and Palate/Ectodermal Dysplasia Syndrome is Identified

*Background:* Cleft lip/palate is one the most common birth defects and yet the underlying causes of this birth defect are largely unknown. Some of these defects are the result of mutations of single genes involved in facial development.

*Advance:* One form of cleft lip palate is an autosomal ("non-sex" chromosome) recessive syndrome (CLPED1), that is, a condition in which an abnormal gene on one of the chromosomes from each parent is required to cause the disease. The syndrome also involves ectodermal dysplasia ( a congenital defect of the ectodermal tissues, including the skin and teeth, and related structures), developmental defects of the hands, and, in some cases, includes mental retardation A gene (PVRL1) has been identified which is responsible for this syndrome. It encodes a protein which is important in cell-cell adhesion. PVRL1 mutations lead to truncated proteins that prevent normal development.

*Implications:* The identification of this gene advances our understanding of the processes involved in orofacial development and of the mechanisms underlying cleft lip/palate. Future studies of the role of this protein, and its interactions with other genes associated with cleft lip/palate will also help us to better understand cell adhesion.

Suzuki K, Hu D, Bustos T, et al: Mutations of PVRL1, encoding a cell-cell adhesion molecule/herpesvirus receptor, in cleft lip/palate-ectodermal dysplasia. Nature Genetics, 25(4):427-30. 2000.

## Gene for Autosomal Hypohidrotic Ectodermal Dysplasia is Identified

*Background*: The skin and associated structures (including hair, nails, sweat glands, and teeth) are derived from the outer layers of cells in the embryo, the embryonic ectoderm. Hypohidrotic ectodermal dysplasia is a disorder in the formation of teeth, hair, and sweat glands. It occurs as both sex-linked and non-sex linked genetic disorders.

*Advance*: In 1996, the gene ED1 was identified for the major X-linked forms of the disease. This year, the gene (DL) for the autosomal forms was mapped to chromosome 2. DL was found to contain mutation in individuals with the disorder. The discovery was accelerated by the previous identification of an analogous mouse gene responsible for a similar disorder. The DL protein may act as the receptor, a protein molecule on the cell surface that binds to a specific factor, for the signaling protein ED1.

*Implication:* A third, as yet unidentified gene, is being sought since it may play a role in ectodermal dysplasia for those individuals who do not contain a mutation in the DL or ED1 genes. Discovery of the DL gene will aid in the understanding, diagnosis and future development of therapies for this disorder.

Monreal A, Ferguson B, Headon D, et al: Mutations of the human homologue of mouse dl cause autosomal recessive and dominant hypohidrotic ectodermal dysplasia. Nature Genetics, 22(4):366-9. 1999.

## Changes in a Viral Surface Protein Predict Outcome of Hepatitis C Infection

*Background:* A major cause of chronic liver inflammation, cirrhosis, and liver cancer, hepatitis C virus (HCV) infection is a severe public health problem worldwide. Nearly 4 million Americans have been infected with HCV, and 8,000 to 10,000 deaths in the U.S. each year are attributable to the infection. This primarily blood borne infection is responsible for half of all liver transplants. The course of disease in HCV-infected patients is highly variable. In about 15 percent of all cases, the infection resolves after a short time. In almost three-quarters of the patients who develop chronic infections, the disease remains mild and relatively stable for decades. For the remainder, however, HCV disease progresses rapidly. A small fraction develop fulminant hepatitis, the most severe form of disease in which large portions of the liver become necrotic (die off). Fulminant hepatitis frequently results in organ failure. Chronic HCV infection cannot be eradicated through currently available therapies, nor can the severity of its clinical course be predicted. Prior to the advance described below, evidence had accumulated that closely related viral variants, defined as quasispecies, might allow HCV to circumvent the immune system. However, there were no data associating the evolution of quasispecies with long-term disease outcomes.

*Advance:* By assessing the DNA sequences of two HCV genes in a group of HCV patients with different clinical manifestations of the disease, and by comparing those sequences, researchers established a correlation between the rate of genetic mutation in HCV and clinical outcome. That is, they were able to show that the extent of mutation of the virus predicts whether HCV will resolve or become chronic. Viruses generally evolve, albeit at varying rates, in response to continuous immune-system attack. The genes that serve as templates for formation of the proteins on the external surface of a virus particle, called envelope proteins, are among the viral genes that mutate the fastest. Using blood serum samples from patients with acute HCV, researchers assessed the number and diversity of mutations in two HCV envelope genes (HCV *E1* and *E2*). When the investigators compared the gene sequences of patients who subsequently cleared the virus with the sequences of those who developed chronic HCV, they found that the HCV in patients who cleared the virus was characterized by evolutionary stasis (lack of mutation). In contrast, the HCV in patients who developed chronic HCV was characterized by genetic evolution. From these results, the investigators conclude that a higher rate of mutation in the HCV envelope genes facilitates evasion of the immune system, leading to chronic disease. The investigators also learned that almost all of the sequence changes occurred within an area known as hypervariable region 1 (HVR1) of the *E2* gene.

*Implications:* This discovery will enable physicians to predict more accurately the clinical course of patients infected with HCV. Moreover, by clarifying the exact protein structure of the altered HVR1 region in the chronic form of the virus, this advance may help further development both of therapeutic vaccines to halt disease progression and prophylactic vaccines that can protect against HVC disease.

Farci P, Shimoda A, Coiana A, et al: The outcome of acute hepatitis C predicted by the evolution of the viral quasispecies. Science, 288(5464):339-44. 2000.

## SCIENCE CAPSULES

**Beta-Endorphin Could Mark Genetic Risk for Alcoholism**. Alcoholism is known to have a strong genetic component, but only some members of the same family will get the disease. Knowing which children are genetically predisposed would be of enormous value, since parents and practitioners could attenuate the risk with counseling and early intervention strategies. Scientists are searching for a collection of biomarkers that, together, will signal an increased risk of alcoholism in specific individuals. They recently added to evidence that the hormone beta-endorphin is a good candidate. The scientists found that the spike in beta-endorphin levels that occurs in some people in response to alcohol consumption is itself a heritable trait, a step toward meeting their criteria for biomarker status.

Froehlich JC, Zink RW, Li T-K, Christian JC: Analysis of heritability of hormonal responses to alcohol in twins: beta-endorphin as a potential biomarker of genetic risk for alcoholism. Alcoholism: Clinical and Experimental Research, 24(3):265-77. 2000.

**Highly Active Antiretroviral Therapy Reverses Brain Metabolite Abnormalities of HIV/AIDS.** In later stages of AIDS, 20% to 40% of patients develop cognitive abnormalities that are referred to as the HIV-cognitive motor complex (HIV-CMC). Patients with HIV-CMC show brain metabolite abnormalities, such as the increased concentrations of myoinositol (MI), a substance that indicates the density of glial cells. The density of glial cells, which support neurons, increases as neurons die. The researchers, hoping to reverse the effects of HIV-CMC and find a way to measure CMC-related brain injury, treated a group of patients with mild HIV-CMC with highly active antiretroviral therapy. They found in assessing the metabolites in the brain through proton magnetic resonance spectroscopy ($^1$H MRS), a brain imaging technique, that there was a normalization of brain metabolites. The levels of MI were reduced by about 12% and there was an increase in T-cell counts. The results indicate that using highly active antiretroviral therapy can reverse brain metabolite abnormalities and raise T-cell counts. Finally, $^1$H MRS can be used to gauge HIV-CMC related brain injury by measuring changes in brain metabolites.

Chang L, Ernst T, Leonido-Yee M, et al: Highly active anti-retroviral therapy reverses brain metabolite abnormalities in mild HIV dementia. Neurology, 53(4):782-9. 1999.

**NIH Researchers Improve Techniques for Interviewing Child Abuse Victims**. A research team has developed new techniques to help police interviewers and child protective service workers obtain more accurate information from victims of child abuse. The new techniques, the first of their kind to translate research findings on child development into concrete procedures and guidelines, rely on asking abused children to recall what happened to them, rather than relying upon cues from the interviewer that may prompt inaccurate answers. This new method will enable interviewers to obtain higher quality

information from children as young as four, which makes it much easier to determine what happened to the children, as well as how to intervene.

Orbach Y, Hershkowitz I, Lamb ME, et al: Assessing the value of structured protocols for forensic interviews of alleged child abuse victims. Child Abuse and Neglect, 24(6):733-52. 2000.

**Type 2 Diabetes in Children, Adolescents and Young Adults**. Type 2 diabetes has traditionally been considered a disease of adults because the age of onset is frequently after age 40 and it is often associated with obesity. Children with diabetes are usually presumed to have type 1 diabetes, an autoimmune disease. In recent years, however, an increasing number of children who appear with elevated blood glucose levels actually have type 2 diabetes. In children, the alarming rise in the incidence of type 2 diabetes appears to be occurring largely in minority populations – Hispanic Americans, African Americans, and Native Americans – and obesity has been shown to be an important risk factor. The mean age at diagnosis is around 13 years, and there appears to be a higher incidence in females. The emergence of type 2 diabetes in this population poses a new clinical problem for the pediatric endocrinologist because not much is known about the therapeutic management of this disorder in childhood. Thus far, children have been treated with a number of different approaches, including diet, oral agents to control blood glucose levels, and insulin. Yet, no scientific data exists to back up these strategies. Research efforts need to be directed towards a better understanding of this disease in childhood, the development of reliable methods for early diagnosis, the development and testing of therapeutic agents for safety and efficacy, and the development of education programs to increase physician awareness. Patient and parent education must also become an integral part of the treatment plan. [secondary – prevention]

Dabelea D, Pettitt DJ, Jones KL, Arslanian SA: Type 2 diabetes mellitus in minority children and adolescents. An emerging problem. Endocrinology and Metabolism Clinics of North America, 28(4):709-29. 1999.

**Determinants of Recovery from Acute Hepatitis C**. Hepatitis C is a common form of viral hepatitis that frequently evolves into a chronic infection that can lead to cirrhosis, end-stage liver disease and liver cancer. The factors of the immune response during acute hepatitis C virus (HCV) infection that lead to recovery as opposed to chronic infection have not been defined. Researchers examined serum samples from patients infected with the same strain of HCV during an outbreak that occurred in the late 1970s. Long-term follow up, as well as samples of serum and T cells (cells used by the body in its fight against infection), were available on a large number of patients 10 to 20 years after onset of the disease. The investigators found that persons who had developed a chronic infection had high levels of antibodies against HCV (B cell, humoral immunity) but had poor HCV-specific T cell responses (cellular immunity). In contrast, patients who had acute hepatitis C in the 1970s, but then recovered, continued to have strong T cell responses to HCV 20 years later, but had low levels of antibody.

Indeed, 42% of recovered patients had no detectable antibody to HCV 20 years after recovery (all had tested antibody positive at 10 years). This study indicates that T cell responses to HCV are long-lived and are perhaps a better biomarker for identifying patients with prior HCV infection and recovery. Furthermore, T cell responses appear to be responsible for clearance of virus and recovery from infection. Thus, vaccines against HCV may need to induce vigorous and long-lived T cell rather than B cell immunity. These insights into immune response will contribute significantly to the design of treatments and vaccines for HCV.

Takaki A, Wiese M, Maertens G, et al: Cellular immune responses persist and humoral responses decrease two decades after recovery from a single-source outbreak of hepatitis C. Nature Medicine, 6(5):578-82. 2000.

**Epidemiology and Mode of Spread of *H. pylori* Infection**. *Helicobacter pylori* is one of the more common infections of man. Worldwide, more than 50% of humans are chronically infected with *H. pylori*. A proportion of infected persons will develop peptic ulcer disease and others develop a severe form of gastritis (atrophic) and/or gastric cancer. The frequency of *H. pylori* infection among Americans and the mode of spread of this disease have not been defined. Researchers have analyzed a large, population based group (NHANES 3) of serum samples from persons in the United States for antibody to *H. pylori* and correlated results with demographic factors including sex, age, race and socioeconomic status. The prevalence of *H. pylori* infection increased with age and was far higher in Mexican Americans (58%) and non-Hispanic blacks (51%) than in non-Hispanic whites (27%). The disparities in frequency of *H. pylori* infection appeared to be related to socioeconomic class and country of origin. The mode of spread of *H. pylori* could not be proven by this cross-sectional study, but the results suggested that poor hygiene and living in crowded living conditions during childhood were associated with a greater likelihood of infection. Researchers investigated the possible mode of spread of *H. pylori*. They admitted *H. pylori*-infected volunteers to a research unit and attempted to culture the organism from oral secretions, air during vomiting, and stool before and again after inducing vomiting or diarrhea. While *H. pylori* was not cultured from these patients' normal saliva or stool samples, it could be cultured from vomitus and from diarrheal stool. Indeed, air-borne distribution of viable *H. pylori* could be demonstrated after episodes of vomiting. This study demonstrates that gastrointestinal infections that cause diarrhea and vomiting may facilitate spread of *H. pylori* in families and thus account for the geographical and socioeconomic-based differences in frequency of this infection. These findings provide a basis for development of recommendations to prevent spread of *H. pylori* in high risk groups.

Everhart JE, Kruszon-Moran D, Perez-Perez GI, Tralka TS, McQuillan G: Seroprevalence and ethnic differences in helicobacter pylori infection among adults in the united states. Journal of Infectious Disease, 181(4):1359-6. 2000.

Parsonnet J, Shmuely H, Haggerty T. Fecal and oral shedding of Helicobacter pylori from healthy infected adults. The Journal of the American Medical Association, 282(23):2240-5. 1999.

**Virus Can Cause Kidney Inflammation**. Kidney inflammation most often occurs from known causes, but can also arise from unknown causes. A recent clinical report identified Epstein-Barr virus (EBV) in the kidneys, but not in other tissues, of patients with chronic kidney inflammation not attributable to known causes. The virus was localized to a specific part of the kidney's functional unit (the nephron) called the proximal tubule; this is the first report of EBV in the proximal tubule. The report suggests that EBV infection may induce a marked immune response and associated inflammation that result in structural damage to the kidney. While EBV infection rarely significantly affects kidney function, the present study suggests that examination of a kidney biopsy for EBV would be an important diagnostic measure that may be used when other causes of kidney disease, as reflected in some loss of kidney function, have been ruled out.

Becker JL, Miller F, Nuovo GJ, et al: Epstein-Barr virus infection of renal proximal tubule cells: possible role in chronic interstitial nephritis. Journal of Clinical Investigation, 104(12):1673-81. 1999.

**Cause of Kidney Stones Mapped to Chromosome 1**. Abnormally high absorption of calcium in the intestine, called absorptive hypercalciuria (AH), is a common cause of kidney stones. Studies of families with a history of kidney stones indicate that an inherited genetic defect is one likely cause of AH. Researchers now have identified a specific region on chromosome 1 that is associated with a severe form of AH. Scientists are pursuing identification of the gene and its mutations, which – when known – may permit early diagnosis of AH and possible prevention of kidney stones.

Reed BY, Heller HJ, Gitomer WL, Pak CY: Mapping a gene defect in absorptive hypercalciuria to chromosome 1q23.3-q24. Jornal of Clinical Endocrinology and Metabolism, 84(11):3907-13. 1999.

**Control of Blood Glucose and Blood Pressure Levels Reduces Diabetes-Related Complications**. The United Kingdom Prospective Diabetes Study, a randomized, controlled clinical trial demonstrated that – compared to conventional management of blood sugar and blood pressure – a policy of intensive control of blood sugar and blood pressure levels substantially reduced the risk of diabetes-related complications in patients with type 2 diabetes. Further analysis of data from this trial now reveals highly significant associations between the development of diabetes-related complications and levels of both systolic blood pressure and blood sugar, as measured by hemoglobin $A_{1c}$ ($HbA_{1c}$) concentration. These associations were found throughout the entire range of blood pressure and blood sugar values typically seen in patients with type 2 diabetes and after correcting for other known risk factors such as age, sex, ethnicity, smoking, and lipid levels. The study also demonstrated that increased blood glucose levels were associated with a greater increase in risk for microvascular complications, such as eye and kidney disease, than that for macrovascular diseases such as heart attack. There was no threshold level of blood sugar or blood pressure below which risk was not increased. These data suggest, therefore, that there is no specific $HbA_{1c}$ or blood pressure target value

for which an individual should aim; rather the closer to "normal," the lower the chance of developing complications. Although not as definitive as a clinical trial analysis, this new observational data suggests that even small improvements in blood sugar and blood pressure control may prevent deaths and other complications due to diabetes.

Adler AI, Stratton IM, Neil HAW, et al: Association of systolic blood pressure with macrovascular and microvascular complications of type 2 diabetes (UKPDS 36): prospective observational study. The British Medical Journal, 321(7258):412-9. 2000.

Stratton IM, Adler AI, Neil HAW, et al: Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35). The British Medical Journal, 321(7258):405-11. 2000.

**Rapid Screening for Cystic Fibrosis Mutations in Nonhuman Primates.** Cystic fibrosis (CF), one of the most common lethal hereditary diseases of Caucasians, may be caused by more than 700 mutations in a particular gene. To evaluate the frequency of naturally occurring mutations, which may be silent disease carriers, scientists have developed a rapid screening technique. Screening more than 1,000 nonhuman primates at the Regional Primate Research Centers revealed a large number of such gene mutations. The rapid screening technique will eventually enable researchers to monitor gene therapy for CF, and the nonhuman primate should provide an excellent model for studying such diseases.

Glavac D, Ravnik-Glavac M, Potocnik U, et al: Screening methods for cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations in non-human primates. European Journal of Physiology, 439(3 Supplement): R12-3. 2000.

**Early Warning of Heart Trouble.** Clinical researchers have discovered that damaged heart tissues step up production of a molecule known as hypoxia-inducible factor 1 (HIF-1) within the first 24 hours of a heart attack or the first 48 hours of acute oxygen starvation (myocardial ischemia). HIF-1, in turn, triggers production a growth factor that stimulates creation of new blood vessels, in an attempt to restore blood flow to the heart. The scientists propose that HIF-1 could serve as an early molecular indicator of progressive oxygen deprivation. And because HIF-1 helps to remedy reduced blood supply, it may also provide clues to treating the damage caused by blocked coronary arteries or conditions that diminish blood flow to the heart.

Lee SH, Wolf PL, Escudero R, et al: Early expression of angiogenesis factors in acute myocardial ischemia and infarction. The New England Journal of Medicine 342(9):626-33. 2000.

**Shining a Light Through the Brain.** Using a thin, flexible headband that contains optical fibers, scientists at the Stanford University General Clinical Research Center safely and noninvasively imaged

changes in brain oxygen levels in adults and critically ill newborns. The headband fibers both emit and detect low-intensity light that travels through brain tissues. Because red and near-infrared light are absorbed by blood, and because changes in blood oxygen levels alter the amount of absorption, the headband technology is able to pinpoint brain regions with fluctuating oxygen levels. Coupled with real-time computer analysis, the imaging technique holds promise as a bedside device for generating continuous, noninvasive brain images that enable diagnosis or monitoring of disease.

Benaron DA, Hintz SR, Villringer A, et al: Noninvasive functional imaging of human brain using light. Journal of Cerebral Blood Flow and Metabolism, 20(3):469-77. 2000.

**HPV DNA Testing in Cervical Cancer Screening.** Previous studies have shown that certain types of human papillomavirus (HPV) cause most cervical cancers, raising the possibility that testing for these HPV types may be a way of screening for the disease. In a study conducted among a high-risk population in Costa Rica, where cervical cancer rates are high, researchers found HPV DNA testing to be a useful screening tool. Compared with conventional Pap testing, in which cervical cells are examined with a microscope, the HPV test was more sensitive (i.e., identified more abnormalities) but less specific (i.e., not as useful in determining the nature or seriousness of the abnormality). These results indicate that HPV testing could be a useful screening tool in cervical cancer prevention programs, particularly in settings in which sensitive detection of high-grade lesions and cancer is paramount.

Schiffman M, Herrero R, Hildesheim A, et al: HPV DNA testing in cervical cancer screening: results from women in a high-risk province of costa rica. The Journal of the American Medical Association, 283(1):87-93. 2000.

**Early Detection of Alzheimer's Disease.** Treatments to slow the loss of nerve cells in progressive neurodegenerative diseases like Alzheimer's and Parkinson's will probably be more feasible than repairing the brain after damage is done. Several such treatment strategies are now emerging. However, very significant brain damage has already occurred before these disorders are detected clinically. Now, scientists using functional magnetic resonance imaging, a type of brain imaging that highlights brain activity rather than anatomical structure, have shown that patterns of brain activity during tasks requiring memory differ depending on genetic risk for Alzheimer's disease. These findings may be useful as an early indicator of memory decline.

Bookheimer SY, Strojwas MH, Cohen MS, et al: Patterns of brain activation in people at risk for Alzheimer's disease, The New England Journal of Medicine, 343(7):450-6. 2000.

**Adult Consequences of Childhood Attention Deficit Hyperactivity Disorder are Common**. The results of this important research suggest that narrow and unrealistic definitions of *remission* used in previous studies of Attention Deficit Hyperactivity Disorder (ADHD) may have led to the overly

optimistic view that ADHD is gone by adulthood – people "grow out of" the disorder. In fact, most people studied with ADHD continue to struggle with a substantial number of symptoms and high levels of dysfunction despite a sizable reduction in the number of symptoms necessary to meet full diagnostic critieria. To clarify the extent to which childhood ADHD persists into adolescence, children with ADHD were followed for 4 years and classified as being in "syndromatic remission" (not meeting full diagnostic criteria for ADHD), "symptomatic remission" (having fewer than the required number of symptoms for the subthreshold diagnosis), or "functional remission" (having symptomatic remission with less than mild impairment). It was found that advancing age significantly affects all forms of remission for ADHD and its subtypes (hyperactivity, impulsivity and inattention); however, the *prevalence* of remission varied considerably. Although *syndromatic* remission was frequent, a more modest *symptomatic* remission was observed, and a very limited functional remission was achieved. This is important because it sheds light on a common misperception that could have direct impact on the treatment of this disorder in adults.

Biederman J, Mick E, Faraone SV: Age-dependent decline of ADHD symptoms revisited: impact of remission definition and symptom type. American Journal of Psychiatry, 157(5):816-8. 2000.

**Measure of Sustained Attention Predicts Vulnerability to Future Schizophrenia Spectrum Conditions.** Schizophrenia is a devastating mental illness, one of the most chronic and disabling of the severe mental disorders. In addition to the development of new treatments, NIH research is focusing on identifying factors that contribute to schizophrenia. NIH investigators have conducted studies leading them to conclude that impaired attention in individuals who later develop schizophrenia-spectrum conditions probably results from prenatal developmental abnormalities on the cellular level, and that it may be a marker of a biological vulnerability to schizophrenia. This study evaluated the role of attentional deficits in the development of schizophrenia and tested the utility of attentional measures for illness screening. Measures of sustained attention (a continuous performance task tapping visual attention) and of behavioral functioning were collected over a 15-year period from 87 subjects at high and low risk for schizophrenia. Attentional deficits were reliably detected in high-risk children who eventually developed schizophrenia-spectrum disorders. These deficits were stable over time, were independent of behavioral difficulties, and were not affected by the onset of illness. Refining measurement of attentional deficits may some day yield a useful screening tool for high-risk youngsters in need of early intervention.

Cornblatt B, Obuchowski M, Roberts S,et al: Cognitive and behavioral precursors of schizophrenia. Development and Psychopathology, 11(3):487-508. 1999.

**Tracing the Course of Prepubertal and Early Adolescent Bipolar Disorder.** Once considered extremely rare, childhood bipolar disorder (manic-depressive illness) is being diagnosed with increasing

frequency in children who also have a diagnosis of attention deficit hyperactivity disorder (ADHD). An ongoing study of prepubertal and early adolescent bipolar disorder (PEA-BP) is examining the naturalistic course of early-onset bipolar disorder in children with or without concurrent ADHD. Ninety-three children (ages 8-13) with a current diagnosis of severe mania or hypomania with elated mood and/or grandiosity were compared to children with ADHD and no manic symptoms as well as normal controls. Compared to both groups, the PEA-BP children had significantly greater impairment on items that assessed maternal-child warmth, maternal-child and paternal-child tension, and peer relationships. Supporting the contention that hypersexuality is a manifestation of child mania, it was found among 43% of the PEA-BP group despite the absence of a history of sexual abuse. At 6-month follow-up, 86% of the PEA-BP children still met full criteria and severity level for mania or hypomania, with elated mood and grandiosity highly stable. These findings are very important because they suggest that PEA-BP may be a stable, valid, and previously under-recognized condition.

Geller B, Zimerman B, Williams M, et al: Diagnostic characteristics of 93 cases of a prepubertal and early adolescent bipolar disorder phenotype by gender, puberty and comorbid attention deficit hyperactivity disorder. Journal of Child and Adolescent Psychopharmacology, 10(3):157-64. 2000.

Geller B, Zimerman B, Williams M, et al: Six-month stability and outcome of a prepubertal and early adolescent bipolar disorder phenotype. Journal of Child and Adolescent Psychopharmacology, 10(3)165-73. 2000.

**Severe Eating Disorders Affect Black American Women.** While it is believed that eating disorders are a more common problem in white than black women, a telephone survey of 1,628 black women and 5,741 white women on binge eating and extreme weight-control behaviors (vomiting, laxative or diuretic abuse or fasting) indicated otherwise. Although both groups reported binge eating at least once during the preceding 3 months, recurrent binge eating, indicative of a clinically severe eating disorder, was reported by significantly more black women than white women. Also, black women were significantly more likely to report fasting, laxative abuse, or diuretic abuse. Regardless of race, women with recurrent binge eating disorder reported significantly more psychiatric symptoms than did control subjects and were overweight. These results challenge the widely held view that black women are immune to developing eating disorders and indicate the potential for correlated physical and mental health problems.

Striegel-Moore RH, Wilfley DE, Pike KM, et al: Recurrent binge eating in black american women. Archives of Family Medicine, 9(1): 83-7. 2000.

**Use of Saliva as a Source of Biomarkers for Cancer Diagnosis.** A prognostic breast cancer marker, c-*erb*B-2 (Her2/*neu*), is assayed in tissue biopsies from women diagnosed with malignant tumors. NIH investigators obtained saliva and serum from three groups consisting of healthy women, women with benign breast lesions and women diagnosed with breast cancer. The salivary and

serological levels of the marker in the cancer patients were significantly higher ($p<0.001$) than the salivary serum levels of healthy controls and individuals with benign tumors. These results suggest that salivary c-*erb*B-2 protein may have potential use in the initial detection (in combination with mammography and physical examination) of breast cancer in women. Due to its ease of collection, a saliva based test could be a non-invasive, cost effective adjunct diagnostic tool.

Streckfus C, Bigler L, Dellinger T. et al: The presence of soluble c-*erb*b-2 in saliva and serum among women with breast carcinoma: a preliminary study. Clinical. Cancer Research, 6(6):2363-70. 2000.

**Standardized Clinical Information Can Predict Conversion to Alzheimer's Disease.** Identification of individuals at high risk of developing Alzheimer's disease (AD) has become an important focus of research. This study identified aspects of a standardized clinical assessment that could predict which individuals with "questionable" AD would have a high likelihood of converting to AD over time. The assessment instrument was the Clinical Dementia Rating (CDR), a clinical interview which stages AD from 0 (normal) to 0.5 (questionable), 1.0 (mild), 2.0 (moderate), 3.0 (severe) based on six categories. After receiving a CDR rating of 0 or 0.5, participants were followed for three years to determine who converted to probable AD. The likelihood of progression to AD during follow-up was related to the Total Box Score, which is the sum of the scores in the six CDR categories. The Total Box Score, combined with 8 selected questions from the clinical interview, identified 89% of those questionable individuals who subsequently converted to AD over the 3 years of the study. These findings are important for offering such patients appropriate counseling, in clinical trial recruitment and in furthering our understanding of the boundary between normal aging and the earliest stages of AD.

Daly E, Zaitchik D, Copeland M, Schmahmann J, Gunther J,Albert M: Predicting conversion to Alzheimer disease using standardized clinical information. Archives of Neurololgy, 57:675-80. 2000.

**Pre-symptomatic Decline in Brain Function in Individuals at Genetic Risk for Alzheimer's Disease**. In individuals with Alzheimer's disease (AD), the APOE-_4 gene is associated with lowered cerebral glucose metabolism in parietal, temporal, and posterior cingulate brain areas, as measured by positron emission tomography (PET) brain imaging. In this study, despite similarities in age, gender, education, family history of dementia, and baseline performance on memory and other cognitive tasks, individuals with APOE-_4 still had significantly lower brain metabolism in several brain regions than non-APOE-_4 carriers at their first exam. After two years these differences widened, as the APOE-_4 carriers showed a 4-5% metabolic decrease in several brain regions compared to the non-APOE-_4 carriers. Moreover, lower baseline metabolism at the start of the study predicted future cognitive decline in memory in subjects at genetic risk for AD. Although longer follow up studies are needed to determine whether the APOE-_4 carriers actually develop AD, these findings indicate that the combination of cerebral metabolic rate and genetic risk factors might be useful as a means for preclinical detection of AD.

Small GW, Ercoli LM, Silverman DHS, et al: Cerebral metabolic and cognitive decline in persons at genetic risk for Alzheimer's disease. Proceedings of the National Academy of Science, 97(11):6037-42. 2000.

**In Vivo Detection of Amyloid Plaques.** To accurately diagnose and monitor the progression of Alzheimer's disease (AD), researchers have been searching for a marker to be used in living patients to identify the senile plaques that are a hallmark of AD and/or the beta-amyloid that comprises the plaques. A new probe called (trans, trans)-bromo-2,5-bis-(3-hydroxycaronyl1-4-hydroxy) styryl-benzene (BSB) has recently been developed. BSB sensitively labels plaques in post mortem AD brain sections and is able to permeate living cells in culture and bind specifically to intracellular beta-amyloid aggregates. After intracerebral injection in living transgenic mouse models, BSB labels plaques composed of human beta-us AD-like plaques throughout the brain of the transgenic mice. This may become the basis for radiological imaging of senile plaques in the brains of living patients that would permit monitoring the progression of AD over time and the clearance of plaques in response to anti-amyloid therapies.

Skovronsky DM, Zhang B, Kung MP, et al: In vivo detection of amyloid plaques in a mouse model of Alzheimer's disease. Proceedings of the National Academy of Sciences, 97(13):7609-14. 2000.

**The Predictive Value of Low Prostate Specific Antigen (PSA) Levels in Older Men.** Over the past 10 years, prostate specific antigen (PSA) testing has become important for the early detection of prostate cancer. However, because of the natural history of prostate cancer, the value of repeated PSA testing may not be equivalent for all men. Scientists therefore hope to optimize the use of PSA for early diagnosis of prostate cancer by identifying populations of men who may be least likely to benefit from repeated PSA testing. Researchers examined men aged 60 to 65 with low PSA levels to determine their likelihood of being diagnosed with prostate cancer by age 75, an age at which the need for surgical treatment would be unlikely. They found that if PSA testing were discontinued in older men whose PSA values are less than 0.5 ng/ml, 100% of the cancers would still be detected by age 75. If a level of 1.0 ng/ml were used as a threshold to stop testing, 94% of cancer cases would still be detected with no further PSA testing. These analyses argue that men who are at low risk for the subsequent development of prostate cancer can be clinically defined, and that continued screening is unnecessary in that population.

Carter HB, Landis PK, Metter EJ, et al: Prostate-specific antigen testing of older men. Journal of the National Cancer Institute, 91(20):1733-77. 1999.

**New Test Proves Useful for Management of AIDS.** Despite the success of treating AIDS patients with combination antiretroviral therapy, the emergence of viral resistance to one or more of the drugs in

the combination remains a problem in some patients. In order to minimize the possibility of a patient progressing from partial to complete drug resistance, scientists have developed an assessment tool called genotypic antiretroviral resistance testing (GART). GART is designed to screen for specific viral mutations that are known to facilitate resistance to specific anti-retroviral drugs. In a recent study, NIH-supported scientists demonstrated that the incorporation of GART in the management of patients doing poorly on combination therapy led to better clinical outcomes than were achieved in a control group in which GART was not used. These results suggest that GART may be useful for early detection and potential retardation of antiretroviral drug resistance.

Baxter JD, Mayers DL, Wentworth DN, et al: A randomized study of antiretroviral management based on blasma genotypic antiretroviral resistance testing in patients failing therapy. CPCRA 046 study team for the terry beirn community programs for clinical research on AIDS. AIDS, 14(9):F83-93. 2000.

**New Test to Diagnose Lyme Disease**. Lyme disease, which is caused by the bacterium *Borrelia burgdorferi*, results is an inflammatory disorder that may effect multiple organ systems. In its early stages, Lyme disease can be treated successfully with oral antibiotics; however, untreated or inadequately-treated infection can progress to late-stage complications requiring more intensive therapy. Early diagnosis is thus essential. NIH-supported investigators have developed a new diagnostic test (the C-6 ELISA test) for Lyme disease. This new test is more effective because it relies on the use of a single highly purified synthetic antigen, rather than an array of uncharacterized bacterial antigens, as is the case with the test currently in use. Another major advantage is that the new test can be used to diagnose individuals who have been immunized with the recently licensed LYMErix$^{(R)}$ vaccine.

Liang FT, Steere AC, Marques AR, et al: Sensitive and specific serodiagnosis of lyme disease by enzyme-linked immunosorbent assay with a peptide based on an immunodominant conserved region of *borrelia burgdorferi* VlsE. Journal of Clinical Microbiology, 37(12):3990-6. 1999.

**Gene Linked to Developmental Syndrome in Old Order Amish**. Scientists at NIH identified an altered gene responsible for a rare, recessive developmental syndrome found predominantly among the Old Order Amish population. Called McKusick-Kaufman syndrome, or MKS, the condition is the first human disorder to be attributed to a mutation in a gene affecting a type of molecule called a chaperonin. The gene on chromosome 20, dubbed MKKS, was altered in a sample from an Amish patient as well as in a sample from a non-Amish patient diagnosed with MKKS. In both people, mutations were found that would alter the protein. Although the function of the protein made by the MKKS gene is unclear, the researchers say that it may be involved in the processing of other proteins in the development of limbs, the heart and reproductive system.

The disorder is most serious in female infants in whom the hydrometrocolpos can cause death because of lung compression complications. It is hoped that a screening process for carrier couples can be

developed so that their pregnancies can be monitored for hydrometrocolpos by ultrasound. Affected girls could be delivered in a setting that would allow rapid surgical correction that could be life saving.

Stone DL, Slavotinek A, Bouffard GG, et al: Mutation of a gene encoding a putative chaperonin causes McKusick-Kaufman syndrome. Nature Genetics, 25(1):79-82. 2000.

**Scientists Pinpoint Location of Possible Third Gene Involved in Hereditary Breast Cancer to Chromosome 13.** Researchers in Finland, Iceland, and Sweden, working with scientists at the NIH, have found evidence of a new gene that appears to increase susceptibility to hereditary breast cancer. The study examined women who live in Nordic countries and who have three or more female family members with breast cancer. The finding may help explain why some women with a family history of hereditary breast cancer are at particularly high risk of developing the potentially fatal disease, even when they lack mutations in two previously identified breast cancer susceptibility genes, BRCA1 and BRCA2.

Initially, spelling errors in the genetic code of BRCA1 and BRCA2 were theorized to account for perhaps 90 percent of all hereditary breast cancers. However, more recent research suggests that these two genes account for a significantly smaller proportion of all hereditary breast cancers. While scientists have not yet identified a third BRCA gene, they have succeeded in pinpointing its probable location to chromosome 13, in an interval of about five million base pairs, a region with an estimated 100 to 150 genes that must be evaluated one by one in order to identify the precise gene responsible for the breast cancer risk.

The possible location of the suspected gene was first identified by applying a technique called comparative genomic hybridization, or CGH, to breast cancer tumor tissues. The tissues came from 61 women with hereditary breast cancer, whose BRCA1 and BRCA2 genes had no mutations. Results from the CGH analysis revealed that genetic material had been deleted in this region of chromosome 13 at an early stage in the development of the tumors, suggesting the presence of a new cancer-causing gene there. Further linkage analysis studies carried out on a larger group of 334 affected women supported the CGH evidence for a new breast cancer susceptibility gene in the same region on chromosome 13.

Kainu T, Juo SH, Desper R, etal: Somatic deletions in hereditary breast cancers implicate 13q21 as a putative novel breast cancer susceptibility locus. Proceedings of the National Academy of Sciences, 97(17):9603-8. 2000.

## STORIES OF DISCOVERY

### New Insights in Assessing the Safety of Insecticides

For some time, there have been questions regarding the possibility that some people might exhibit higher sensitivity to the widely used insecticides Dursban (chorpyifos) and diazinon and their oxygen analogs. A team of NIH-supported investigators has been exploring this suggestion by characterizing one of the enzymes involved in the detoxification of the insecticides and by developing an animal model system to test hypotheses related to these questions.

These investigators studied whether the enzyme paraoxonase (PON1) plays a major role in detoxifying insecticides. The main function of PON1 appears to be protection from vascular disease; however, PON1 has an interesting alternative activity. It inactivates the toxic forms of insecticides as well as some nerve agents such as sarin and soman. If PON1 was discovered to be a major detoxifying agent then the large differences in serum PON1 levels observed between individuals would be relevant to differences in insecticide sensitivity.

A key chemical feature of the insecticides is the attachment of sulfur and phosphorus atoms. Once ingested by organisms, the sulfur atom is replaced by an oxygen atom producing the oxon form of the insecticide that is many times more toxic than the original compound. During manufacturing of the insecticides, other oxons are also formed and more can accumulate after they are applied.

Initial observations by several research groups noted that there were large differences in the rates that serum from different individuals could inactivate the toxic oxon form of parathion, known as paraoxon. These observations led to the concept that individuals with low levels of serum PON1 would be more sensitive to a parathion/paraoxon exposure that people with high levels of PON1. Similar findings were noted in studies of two other insecticides: diazinon and chlorpyrifos. Additional studies provided evidence that high levels of PON1 protect against some insecticides.

The researchers then asked: What is the consequence of having low levels of PONl? This question was answered by determining the sensitivity of mice that were totally lacking PONl (PONl knockout mice). Experiments clearly demonstrated that the mice missing PONl have a dramatically increased sensitivity to chlorpyrifos oxon and to diazoxon and its parent compound diazinon. The availability of the PONl deficient mice allowed the group to inject different isoforms of the purified human PON1 and examine their ability to provide protection against insecticide exposure. The results showed that both isoforms provided equivalent protection against an exposure to diazoxon, however, $PON1R_{192}$ isoform provided better protection against exposure to chlorpyrifos oxon exposure.

Researchers now think that the genetic differences in rates of inactivation of the oxon forms of insecticides observed in the human population probably reflect differences in sensitivity to diazoxon and

chlorpyrifos oxon. In addition, the fact that significant levels of the oxon forms occur in many exposures, leads to the conclusion that one of the major problems with safety testing of these insecticides is that the tests use very pure parent compound, where as real- life exposures include high levels of the highly toxic oxon forms. These data suggest that it is important in safety testing of organophosphate insecticides to include ratios of oxon forms that reflect actual environmental exposures.

How variable is the structure of PONl in human populations? PONl has two DNA sequence changes (mutations) that result in amino acid changes in the protein. The two amino acid mutations are found at positions 55 and 192. These investigators demonstrated that the amino acid present in the 192 position determined which pesticides were best neutralized by PONl. Arginine in this position results in a rapid rate of paraoxon hydrolysis, and glutamine in this position a much slower rate of paraoxon hydrolysis. The opposite is true for sarin hydrolysis. PONl with arginine in this position hydrolyzes sarin very slowly, while PONl with glutamine in this position hydrolyzes sarin much faster. In addition to the effects of the mutation on rates of insecticide inactivation, individuals have different amounts of PONl in their serum. Newborns have very low PONl levels predicting an increased sensitivity to specific insecticide exposures. Thus, depending on the insecticide, the level of PONl and the amino acid present at position 192 can make a major difference in an individual's sensitivity to the specific exposure.

All of the data taken together, leads researchers to the conclusion that there are indeed individuals who exhibit much higher than normal sensitivity to diazinon, chlorpyrifos, and their oxon forms. At risk individuals include very young children and adults with low serum PONl concentrations. Scientists also now know that a significant contributor to the toxic effects of the exposure is the level of oxidized insecticide (oxon form) present in the exposure. This research underscores the important concept that safety testing of this class of insecticides should include studies on direct exposure to oxon levels comparable to those actually experienced in real-life exposures.

## Why the Kidney Sometimes Leaks Protein – Studies of Cells with Feet

Normal kidneys work very efficiently to cleanse the blood of waste products and retain normal blood constituents – water, salts, and blood proteins. The first step in this process is filtration of the blood plasma by the renal glomerulus, a complex structure consisting of a tiny ball of delicate capillaries surrounded by special cells called *podocytes*. The human kidney has a million of these tiny filters. Massive quantities of fluid are filtered from the blood by the glomeruli, about one hundred and eighty liters per day, but very little protein escapes. Blood proteins do not leak out because the filtration properties of the glomerulus result in retention of large molecules such as albumin and other proteins. The podocyte – meaning cell with feet – is a critical component of this filter. New evidence establishes that it plays the major role in synthesizing the scaffold that lets fluid through and holds back blood proteins; it is the key to maintaining the integrity of the filter.

Physicians have long been aware that leakage of protein into the urine is an important early sign of kidney disease, but the significance of even small amounts of protein in the urine as a risk factor for eventual kidney failure has only recently been appreciated. In the last few years, several large clinical trials have examined the progression of kidney dysfunction to kidney failure; it has been found that patients with small amounts of protein in the urine are much more likely to progress to kidney failure than other patients who have equal degrees of kidney dysfunction but who do not have protein in the urine. In patients with diabetes, the earliest warning for the presence of kidney involvement is the appearance of small amounts of albumin in the urine. Recent studies show that reduction in the amount of albumin in the urine is associated with stabilization of disease, and increases in albumin and other urine proteins are linked to development of kidney failure.

Another important condition characterized by protein in the urine is the nephrotic syndrome. The end result of a variety of diseases, this condition is characterized by massive losses of blood proteins into the urine. Loss of large amounts of protein in the urine causes a variety of disturbances in body function, including retention of salt and water, high blood pressure and high cholesterol. One of the commonest forms of nephrotic syndrome occurs in children. In some rare cases, it runs in families. Currently, treatment of nephrotic syndrome focuses on identifying the underlying cause, if possible, and reducing high cholesterol, blood pressure, and protein in the urine through diet, medications, or both. One group of blood pressure medications, known as ACE inhibitors, which also protects the kidneys of diabetic patients, are sometimes helpful for reducing proteinuria in nephrotic syndrome, but in many cases the proteinuria is resistant to treatment. All these observations underscore the importance of understanding the glomerular filter and learning what holds back proteins in the glomerulus and what causes leakiness.

Over several decades, basic research on the kidney glomerulus has yielded a clear description of the filtration barrier. We know that the filter is composed of three layers: the cells lining the capillaries (endothelial cells), the podocytes (the cells with feet) and the glomerular basement membrane, a

collagenous gel that separates the two cell layers. Stretching between the "feet" of the podocytes is the "slit diaphragm," the principal filtration barrier to large blood proteins.

The first genetic defect established to cause leak of proteins into the urine was in the collagen molecules that make up the glomerular basement membrane. Defects in this form of collagen cause Alport's syndrome, a disorder in which protein leaks into the urine, and kidney failure may develop. Kidney biopsies from patients with Alport's syndrome show very thin glomerular basement membranes. In most conditions where there is protein in the urine, however, the glomerular basement membranes appear structurally normal.

Increasingly, research interest has focused on the podocyte and on understanding the special molecules that the podocyte uses to form the slit diaphragm and maintain the filtration barrier. In the past three years there has been a rapid series of important discoveries in understanding the molecular basis of podocyte function – and malfunction. These insights have emerged primarily from the application of genetic approaches to either man or mouse, but they have been helped by decades of studies in rats and insights from the genome of the roundworm.

The first of these observations occurred in 1998, when scientists found the gene mutated in a rare type of hereditary nephrotic syndrome found in Finnish families. The gene's protein product, called nephrin, was found to be expressed only in the podocyte and to be a major component of the slit diaphragm filtration barrier.

In 1999, a second group of investigators discovered, unexpectedly, that massive proteinuria and kidney failure developed in mice that were altered genetically to lack a protein named CD2 adaptor protein (CD2AP), a protein not previously known to be important in kidney function. In fact, prior to these studies, CD2AP was thought to function only as a T lymphocyte protein and part of the immune system. The researchers found that, in fact, CD2AP interacts with nephrin in the slit diaphragm, and suggested that CD2AP may serve to anchor nephrin to the podocyte.

Then, in March 2000, scientists reported the discovery – again using genetic approaches – of a third protein, named a-actinin-4, that is produced by a gene located close to the nephrin gene. Mutations in this gene cause a kidney disease called focal sclerosis that leads to nephrotic syndrome and kidney failure in adulthood. The investigators showed that mutant a-actinin-4 actually binds more strongly to a protein in the podocyte that helps maintain the shape of the cell's foot processes than does a-actinin-4 from non-affected individuals. The mutant α-actinin-4 also is produced at higher levels in podocytes. They speculate that altered a-actinin-4 may act in a dominant fashion to alter podocyte foot shape, thereby altering the structure of the slit diaphragm and glomerular function, resulting in slow accumulation of kidney damage.

Following these observations in close succession was another report in April 2000, of a fourth gene which, when mutated, causes protein in the urine. The gene produces a membrane protein, named podocin, and again, it is exclusively expressed in the podocyte. It was found to be defective in thirteen families with a form of nephrotic syndrome that presents in infancy and leads rapidly to kidney failure. While the precise function of podocin is not yet known, it is very similar to a protein called MEC-2 found in the roundworm, *C. elegans*. MEC-2 functions to link stretch-sensitive ion channels on the cell surface with internal skeletal structures of the cell. Thus, these investigators suggested that podocin may be important for cell-surface interactions critical in maintaining the shape of podocyte foot processes. This research is an example of how detailed knowledge of the genome of a simpler, non-mammalian model system has helped to accelerate the pace of human disease research. In the case of *C. elegans*, investigators will be able to perform studies of the podocin-like protein to define its interactions with other proteins. This information will bear directly on the study of the filtration barrier in humans.

Studies of these four proteins – nephrin, CD2AP, a-actinin, and podocin – are revolutionizing our fundamental knowledge of the molecular mechanisms of glomerular filtration. As abnormal function of the filtration barrier is a major complication in most clinically important kidney diseases, such as hypertensive nephropathy and diabetic kidney disease, further studies of these proteins has great promise to suggest new strategies for treatment and prevention.